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PHOSPHOGLUCOSE ISOMERASE VARIABILITY IN TWO SYMPATRIC SPECIES OF OSTREIDAE IN THE COASTAL LAGOON MAR MENOR (SE SPAIN)

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RESUMEN

En la laguna costera del Mar Menor (SE de España) coexisten dos especies de ostras. Ambas especies son morfológicamente similares y sólo se diferencian en su talla máxima; *Ostreola stentina* (Payraudeau, 1826) crece hasta los 5 cm de longitud máxima y *Ostrea edulis* (Linné, 1758) hasta los 11-12 cm. Esta similitud morfológica entre ambas especies provoca algunos problemas en el cultivo de la *Ostrea edulis*. Sin embargo, las frecuencias alélicas del *locus* de la *GPI* pueden ser usadas como carácter diagnóstico de las especies.

También se compara la variabilidad genética de ambas especies en el Mar Menor. *Ostrea edulis* tiene altos niveles de homocigosis y *Ostreola stentina* presenta una mayor heterocigosis.

Palabras clave: *Ostrea*, diferenciación genética, enzimas, cultivo marino, Mar Menor, España

ABSTRACT

Two species of oysters coexist in Mar Menor coastal lagoon (SE Spain). Both species are very similar and are only differentiated on the size; *Ostreola stentina* (Payraudeau, 1826) which growth until five centimeters of maximum length, and *Ostrea edulis* (Linné, 1758) until 11 or 12 cm. This morphological similarity causes some problems in the hatchery of *Ostrea edulis*. The allele frequencies at the *GPI locus* can be used as species-diagnostic character.

The genetic variability of the glucose-6-phosphate isomerase (*GPI*) *locus* is also compared in *Ostrea edulis* and *Ostreola stentina* from Mar Menor. *Ostrea edulis* shows high levels of homozigosity and *Ostreola stentina* has greater heterozigosity than *Ostrea edulis*

Key words: *Ostrea*, genetic differentiation, enzymes, hatchery, Mar Menor, Spain

1. INTRODUCTION

Oysters have been exploited since the time of the Roman Empire (MAGENIS *et al.* [14]), but the harvesting on a large scale began in France about 1850 (JAZIRI *et al.* [11]). Aquaculture productivity cannot be optimised if the biological potential of the cultured species is not realised. Individuals that have faster growth rates lower food conversion and higher survival are more economical to raise (TORO & NEWKIRK [3]).

In the Mar Menor the spats of two oyster species (*Ostrea edulis* and *Ostreola stentina*) have been collected from natural beds and cultured with varying success due to epizootic diseases and irregular growth of a percentile individuals.

Ostrea edulis has a wide geographical distribution along the Atlantic coasts from Norway to Morocco, and along the Mediterranean and Black Sea coasts (YONGE [28]). It is a hermaphrodite species that can be found in subtidal habitats. Its life history is characterised by fertilisation occurring inside the pallial cavity and brooding of the larvae (YONGE [28]). As a result of a brooding period of 8 to 10 days, the length of the plankton larval phase is reduced compared to that of the other oyster species (BUROKER [5]). *Ostrea edulis* have a high commercial value and its populations have suffered strong declines due to overexploitation (YONGE [28]).

Ostreola stentina have small to medium size and its shells are irregularly subcircular to somewhat dorso-ventrally elongate, this specie lives in shallow subtidal waters to a few meters depth, in tropical and temperate seas (HARRY [10]).

The Mar Menor is a coastal lagoon located at the SE of Spain whose waters are hypersaline and oligotrophic. It has five open canals what permit the interchange with Mediterranean Sea. The most important of them, the Estacio Channel, was dredged in 1970´s provoking important changes at the hydrodynamism and communities of the Mar Menor. Some species original of Mar Menor disappeared and other ones from Mediterranean Sea poured into coastal lagoon, as *Ostrea edulis*. This specie had a fast expansion in the Mar Menor. In 1992, 177 millions individuals were accounted with an average density of 1,986 oysters/m^2 and densities of 22 oysters/m^2 in the most populated areas (ROSIQUE & GARCÍA-GARCÍA [20]).

Because of these high densities several attempts of hatchery were done, but the low growth rate of a percentile individuals did not permit a profitable use. BLANC *et al.* [3] already cited a similar chance in Nadoor lagoon (Morocco). They studied two populations of oysters what belong to same cohort: 49 individuals of normal growth and 49 individuals of slow growth. They concluded that the fast-growing sample was *Ostrea edulis* and the slow-growing oysters: 19% only was considered as *Ostrea edulis* while 81% belong to another species. Moreover this second specie differed from *Ostrea edulis* by three *loci* and appeared as a dwarf sibling species of *Ostrea edulis* with similar larvae and spat.

Systematic position of *Ostreidae* has been studied in several works (PASTEUR-HUMBERT [19], BORN [4], GMELIN [7], ORTON [17], NELSON [16], MONTERO [15], STENZEL [24], PARENZAN [18]) but most of them have not resolved the taxonomic problem. HARRY [10] presented a good synopsis of the supra-specific classification of living oysters where he considered not only the structure of the flesh and shells but also the environments, geographic range, and behaviour of oysters. The author concluded that intra-specific variation of oyster shells, which is probably greater than in any other group of living bivalves, precluded the preparation of a simple and satisfactory taxonomic key.

Oyster populations inhabiting the Mar Menor show these difficulties. The study of morphological characters do not reveal any differences (larval phase and spats are very similar).

In the last years, population genetics has been used to solve many speciation problems in marine organisms. Abundant and ecologically important 'species' have been in fact groups of species or species complexes and this has been demonstrated mainly using biochemical methods (AVISE, [2]).

Variation in enzyme coding genes has been studied in recent years in several species of marine bivalves; it has provided the differentiation among similar species and information about genetic structure of populations of these organisms.

Several studies of variations at enzyme *loci* have been made at *Ostrea edulis* (WILKINS & MATHERS [27], BUROKER [6], MAGGENIS *et al.* [14]; JOHANNESSON *et al.*[12], LE PENNEC *et al.*[13], BLANC *et al.* [3], SAAVEDRA *et al.* [21],[23], ÁLVAREZ *et al.* [1]. Electrophoretic studies have been mainly restricted to the Atlantic populations, which have been very much affected by human harvesting activities (YONGE [28], MAGGENIS, *et al.* [14]. These studies indicated a great genetic uniformity, covering restricted areas of the total range of the species distribution (LE PENNEC *et al.* [13], JAZIRI *et al.* [11], SAAVEDRA *et al* [21]..

In this paper, genetic variation at the *PGI* is compared in *Ostrea edulis* and *Ostreola stentina*, and evidence is presented which indicates some alleles at the *PGI locus* can be used as species-diagnostic character with a high probability of distinguishing these two species.

2. MATERIALS AND METHODS

Sampling

In order to confirm the existence both species in the coastal lagoon, to determinate its importance and relate them with the growth in the installations of hatchery, three samples were collected at 1996 (Figure 1).

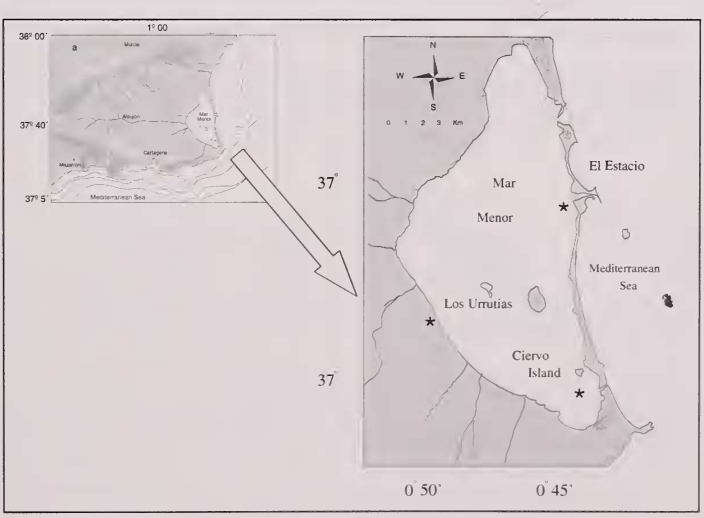

Figure 1. Localities where *Ostrea edulis* and *Ostreola stentina* were sampled.

The first sample were collected as spat in El Estacio (Mar Menor) in January and moved to Marbella where 8 months later were collected as adult oyster (around 110 individuals). Other samples were taken from Los Urrutias and Ciervo island (Mar Menor) which are natural oyster beds. The oysters were transported to the laboratory where they were dissected.

Morphometry

Three measures were made on the shells: maximum shell length (L1), shell height (maximum dimension in the plane of symmetry perpendicular to shell length, L3), and shell width (maximum dimension perpendicular to the plane of symmetry, L2).

Electrophoresis

Portions of abductor muscle were removed from each individual, homogenised in 1.5M Tris buffer (pH 9), and centrifuged at 4ºC and 13500xg. They were stored at -40ºC until electrophoresis.

Vertical polyacrilamide gel electrophoresis was carried out at constant voltage (125 V) for 5 hours at 4ºC. Gels were stained for *PGI* activity as described in HARRIS & HOPKINSON [9] with some modifications in the proportions of reactives (see GONZÁLEZ-WANGÜEMERT [8].

Isozymes were numbered in decreasing order of mobility starting from the most anodal; allozymes were encoded according to the mobility of the most common allele (100).

The genetic variability of the samples was recorded as expected and observed heterozygosity (He and Ho respectively).

3. RESULTS

The morphological characters did not discriminate between both oyster species. They only are differentiated in their size. An overlapping is showed between both species (smaller size classes) presenting similar biometrics relationships though they show different

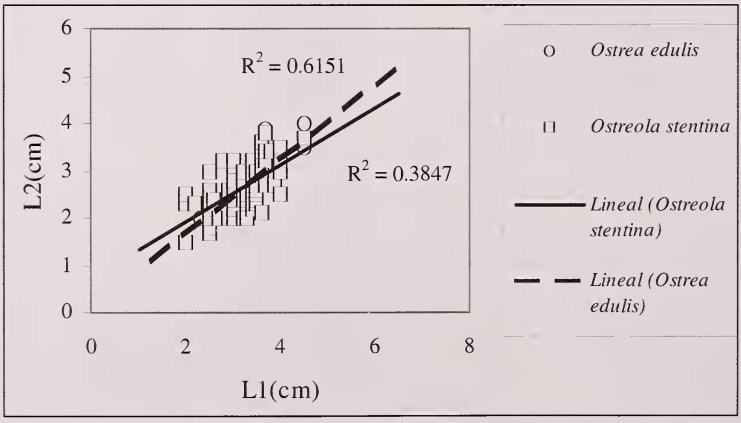

Figure 2. *Ostrea edulis* (○) and *Ostreola stentina* (□). Biometric relationships (L1-L2).

regression coefficients (r^2=0.6151 *Ostrea edulis* and r^2=0.3847 *Ostreola stentina*), so that they can not differentiated about these morphological characters (Figure 2).

Similar results are obtained when the absolute frequencies of size class are represented (Figure 3). An overlapping is showed between 2-5 cm of size for two species.

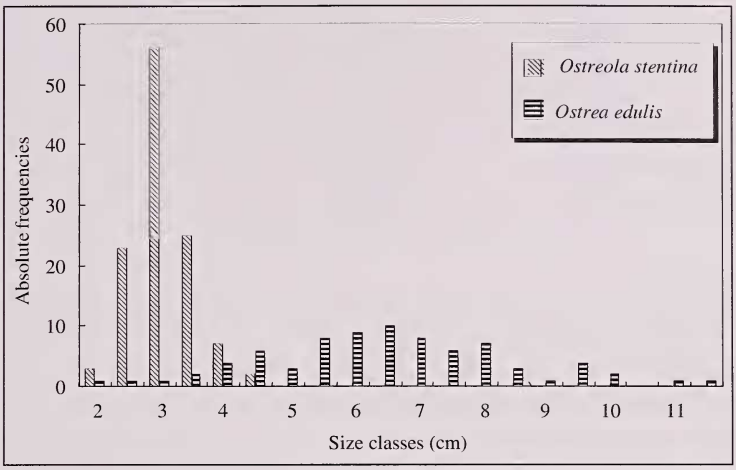

Figure 3. *Ostrea edulis* and *Ostreola stentina.* Absolute frequencies of size class.

Glucose-6-phosphate isomerase was encoded by three alleles in *Ostrea edulis* (Table I), though only three individuals exhibited the *PGI**115 and one individual show the allele *PGI**110 in heterozygous combination (Table II). The *locus* can be regarded as essentially monomorphic in this specie.

Table I. Allele frequencies at *GPI locus* of *Ostrea edulis* and *Ostreola stentina.*

Species	Locality	*PGI 70**	*PGI 85**	*PGI 95**	*PGI 100**	*PGI 110**	*PGI 115**
Ostrea edulis	Estacio-Marbella	0	0	0	0.95	0.03	0.03
Ostrea edulis	Ciervo Island	0	0	0	1	0	0
Ostrea edulis	Urrutias	0	0	0	0.97	0	0.03
Ostreola stentina	Estacio-Marbella	0.26	0.64	0.09	0	0	0
Ostreola stentina	Ciervo Island	0.39	0.57	0.05	0	0	0
Ostreola stentina	Urrutias	0.38	0.46	0.15	0	0	0

In *Ostreola stentina* three alleles of the phosphoglucose isomerase were expressed with frequencies higher than 0.1000, so that the *locus* can be regarded polymorphic.

Table II. Relative frequencies of *GPI* genotypes in *Ostrea edulis* and *Ostreola stentina*.

Genotypes	*Ostreola stentina*	*Ostrea edulis*
**100/100*	0	0.9452
**100/115*	0	0.0410
**100/110*	0	0.0136
**70/70*	0.0930	0
**70/85*	0.3813	0
**70/95*	0.1101	0
**85/85*	0.3644	0
**85/95*	0.0762	0
**95/95*	0.0084	0

Low differences were observed between the observed and expected heterozygosity. The highest deviation coefficient (D) was 0.0278 to *Ostrea edulis* and 0.111 to *Ostreola stentina* (Table III). The observed heterozygosity in *Ostrea edulis* showed low values (ranged from 0.00 to 0.0952) because the *PGI**100 is mainly combined as homozygote and only four individuals were heterozygotes. *Ostreola stentina* has higher observed heterozygosity than *Ostrea edulis*, because 56 percent of analysed individuals were heterozygotes.

Table III. Observed and expected heterozygosities (Ho and He respectively) and deviation coefficient (D).

Species	Locality	Ho	He	D
Ostrea edulis	Estacio-Marbella	0.0952	0.0963	-0.0106
Ostrea edulis	Ciervo Island	0	0	0
Ostrea edulis	Urrutias	0.0556	0.0540	0.0278
Ostreola stentina	Estacio-Marbella	0.5000	0.5066	-0.0132
Ostreola stentina	Ciervo Island	0.5909	0.5258	0.1101
Ostreola stentina	Urrutias	0.6923	0.6154	0.1111

4. DISCUSSION

Both species are very similar, biometric relations do not allow us to differentiate the species for small class of size because there is an overlapping. The preliminary genetic data allow us to differentiate two oyster species using the allele frequencies at the *GPI locus* as species-diagnostic character. The coexistence of both species could explain the disaster of the oysters hatchery attempt in the Mar Menor.

Genetic variation within and between populations has been demonstrated by the use of electrophoresis. Now we have some information about the frequencies and distribution of alleles in the wild populations of *Ostrea edulis* and *Ostreola stentina* in the Mar Menor. Lower levels of genetic variation are detected in the population of *Ostrea edulis*. All the electrophoresis studies of *Ostrea edulis* populations agree in this species display lower levels of allozyme variation than other bivalves (BUROKER [5], SAAVEDRA *et al.*[21]

and the overall differentiation among its populations is usually slight (JOHANNESSON *et al.* [12]. In fact SAAVEDRA *et al.* [22] show through an UPGMA dendrogram based on Nei's unbiased genetic distances (D), an important relationship among Marsella and Mar Menor populations. This dendrogram produced two main clusters, one formed by the eastern Mediterranean samples and the other by the remaining populations (western Mediterranean and Atlantic samples).

Populations of *Ostrea edulis* have an observed heterozigosity very low, though important deficit of heterozygotes is not observed. Some researches have documented deficit of heterozygotes for populations of *Ostrea edulis* from Atlantic oyster beds (BUROKER [5], MAGGENIS *et al.* [14], JOHANNESSON, *et al.* [12]. The biological origin of these deficiencies of heterozygote genotypes may be related to fertilization. It takes place inside the pallial cavity of the female, which favours mating between, nearest-neighbours, and larvae are brooded for a period of 8 or 10 days before the plankton phase.

Low levels of variation were not evident in population of *Ostreola stentina*. This specie show six different genotypes for phosphoglucose isomerase and high observed heterozygosity (Ho= 0.5447). This high variability could be due to long larval period (HARRY [10]) which could favours the dispersion of gene pool. Some authors affirm that patterns of variability at the *PGI locus* in bivalves suggest that species inhabiting temporally variable or spatially heterogeneous environments exhibit higher levels of genetic variability than those from less variable or more monotonous environments (VALENTINE & AYALA [26]).

The Mar Menor is a habitat with environmental parameters very variable, so that this fact can explain the high genetic variability in *Ostreola stentina.* However in the population of *Ostrea edulis* the low levels of allozyme observed variation may be dued to the recent history and exploitation of the populations. Nowadays oyster bed of *Ostrea edulis* in the Mar Menor come from oyster beds harvested for commercial purposes in NW of Spain.

The results of this study confirm that there are two species (*Ostrea edulis* and *Ostreola stentina*) in the stock of Mar Menor and the allele frequencies at the *PGI locus* can be used as species-diagnostic character.

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